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A new species of *Loxosceles* (Araneae, Sicariidae) from Tunisia

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Abstract

A new species of the spider genus *Loxosceles*, *L. mrazig* **sp. n.**, found in Tunisia is described and illustrated. The male bulb shows a high degree of morphological similarity to that of *L. gaucho* from Brazil, but the proportions of the palpal segments and the general colouration of the body reveal significant differences between the two species. A distance analysis of the sequences of the mitochondrial gene *cox1* reveals that the specimen from Tunisia shows high genetic distance from *L. gaucho* (more than 20%). The American species *L. gaucho* and *L. laeta* form a sister group to the Mediterranean representatives (*L. rufescens* and the Tunisian specimen).

Keywords

Taxonomy, Araneae, *Loxosceles*, new species, Tunisia

Introduction

The genus *Loxosceles* Heineken et Lowe, 1832 is currently known to comprise 97 species (Platnick 2009), 82 of which occur in America, 12 in Africa and two in China. Following Brignoli's (1969, 1976) contributions with respect to the Mediterranean basin, only a single species is currently accepted as valid, *L. rufescens* (Dufour, 1820),

whose type locality is near Sagunt, Valencia (Spain). The other two (sub-) species are considered *nomina dubia*: *L. decemnotata* Franganillo, 1925 from Spain and *L. rufescens lucifuga* Simon, 1910 from Algeria. In the same paper Brignoli (1976) reported the South American species *L. gaucho* Gertsch, 1967 from Tunisia.

In 2007 colleagues from the Ecology Department at the University of Barcelona collected in Douz (Tunisia) a male of *Loxosceles* in a dune located several kilometres from the city. The morphology of the copulatory bulb is remarkably similar to that of *L. gaucho* from Brazil, although the differences of the proportions of the male palpal segments plus the general colouration of the body suggested that it could be a different species. In order to test this curious distribution, we used the cytochrome oxidase I gene (*cox1*) to compare this new record with *L. gaucho* (Sao Paulo, Brazil).

Material and methods

Taxonomy. Specimens were examined under a Zeiss Stereo Discovery V12 stereomicroscope equipped with an Infinity X DeltaPix digital camera. Digital microscopic images were edited using DeltaPix DpxWiew Pro AZ V. 13.6 software, using an enhanced focus function. Ink drawn digital illustrations were generated with the assistance of Photoshop CS3 software.

Measurements were taken using the enhanced focus function incorporated into the DeltaPix DpxWiew Pro AZ software. All morphological measurements are given in millimetres. Prosoma and opisthosoma measurements were taken in dorsal view. Total body length represents the sum of the lengths of the prosoma and opisthosoma, omitting the pedicel. Eye largest diameters were taken from the spans of the lens. The largest leg article lengths were measured in lateral view without detaching the legs from the specimen, by placing the article being measured in a perpendicular position. Holotype, and all other specimens are deposited in the Arachnid Collection of the CRBA (Centre de Recursos de Biodiversitat Animal) at the University of Barcelona; catalogue numbers are given in brackets.

Abbreviations used in the text. CRBA – Centre de Recursos de Biodiversitat Animal, Universitat de Barcelona, Spain. Eyes: **ME** = median eyes; **LE** = lateral eyes.

Molecular data. Taxonomic sampling. Taxa analyzed in the present study are listed in Table 2. *L. mrazig* sp. n. from Douz (Tunisia), *L. gaucho* from Sao Paulo (Brazil) and nine specimens of *L. rufescens* from different localities in the Iberian Peninsula and Tunisia were analyzed. In addition we included a representative of *Loxosceles laeta* (Nicolet, 1849) (Montevideo, Uruguay) in order to test the phylogenetic affinities of *L. gaucho* with other South American species, since *L. laeta* belongs to a different species group (Gertsch 1967; Binford et al. 2008). A sequence from *Dysdera crocata* C. L. Koch, 1838 from GenBank was also included to root the tree.

Sample Storage and DNA Extraction. Specimens were preserved in 95% or absolute ethanol and stored at 4°C. Total genomic DNA was extracted from legs of a single specimen using the QIamp® DNA Mini Kit (QIAGEN) following the manufacturer's

protocols. The approximate concentration and purity of the DNA obtained were verified using 1.5% agarose/TBE gel electrophoresis.

PCR Amplification and Sequencing. A total of 899 bp of the cytochrome oxidase I gene (*cox1*) was amplified from each individual using PCR with the following primer pairs: C1-J-1718 (Simon et al. 1994) with C1-N-2776 (Hedin and Maddison 2001). The PCR reaction mixture contained a final concentration of 0.2 µM of each primer, 0.2 mM of each dNTPs, 0.5 U Taq polymerase (Promega), with the supplied buffer, and 1.5-2.5 mM Mg Cl2 in a final volume of 25 µL.

A Perking-ElmerCetus Moldel 480 thermocycler was used to perform 35 iterations of the following cycle: 30s at 94°C, 45s at 44°C, and 1 min at 72°C, beginning with an additional step of 3 min at 94°C, and ending with another step of 5 min at 72°C. The PCR results were visualized by means of a 1.5% agarose/TBE gel. Amplified products were purified using MultiScreen 96 – well filter plates from Millipore. The purified products were directly cycle-sequenced from both strands using ABI BigDye (Applied Biosystems) chemistry and run out on ABI Prism 377 (Applied Biosystems) automated sequencers. Sequencing reactions were performed in our lab with the forward and reverse PCR primers and one additional pair of internal *cox1* primers, CI-J-2183 and C1-N-2191 (Simon et al. 1994). The resulting products were run and analyzed at the Serveis Científico-Tècnics of the Universitat de Barcelona.

Alignment. Raw sequences were compared against chromatograms and complementary contigs built and edited using the Geneious Pro 3.6.2 software (http://www.genious.com). Sequences were manipulated and preliminary manual alignments constructed using BioEdit V.7.0.5.3 (Hall 1999). Alignment of *cox1* was trivial, given that no evidence of insertions/deletions was observed.

Genetic distances and distance analyses. Uncorrected genetic distances between and within taxa were estimated with MEGA v.3.0 (Kumar et al. 2004). The Neighbour-joining algorithm was applied to the estimated genetic distances to build a phenogram (Saitou and Nei 1987) conducted with the same program. Clade support was assessed via Bootstrap (Felsenstein 1985) as implemented in MEGA, based on 1000 bootstrap replicates.

Results. Molecular data. The distance tree is shown in Fig. 1. The specimens identified as *L. rufescens* form a monophyletic clade with a high support value (100%). *L. mrazig* sp. n. is supported as more closely related to *L. rufescens* (75% bootstrap value) than to the two South American representatives included in this analysis: *L. gaucho* and *L. laeta*. The latter two species cluster together with moderate support (68%).

Averages between group genetic distances are presented in Table 1. The cluster formed by the nine specimens of *L. rufescens* from Spain and Tunisia shows scarce

Table 1. Average between group genetic distances of gene *cox1* from the four species analyzed.

	L. rufescens	***L. mrazig***	***L. gaucho***
L. mrazig	0.1991		
L. gaucho	0.1927	0.2063	
L. laeta	0.1973	0.2086	0.1635

within-group average genetic distances (0.26%) and suggests that this species shows a high genetic coherence. The deep genetic divergence between *L. mrazig* and *L. gaucho* (20.63%) together with the observation that both species belong to different clusters provide clear evidence that *L. mrazig* is an independent evolutionary lineage and should, therefore, be considered a different species.

Table 2. Species included in the phylogenetic analysis and GenBank accession numbers for *cox1*.

Species	Locality	GenBank Accession number
Loxosceles laeta	Montevideo, Uruguay	FJ986177
Loxosceles gaucho	Sao Paulo, Brazil	FJ986178
Loxosceles mrazig sp. n.	Douz, Tunisia	FJ986179
Loxosceles rufescens	Torrejon de Ardoz, Madrid, Spain	FJ986183
Loxosceles rufescens	Ciudad Real, Spain	FJ986185
Loxosceles rufescens	Denia, Alacant, Spain	FJ986187
Loxosceles rufescens	Chumilla, Murcia, Spain	FJ986181
Loxosceles rufescens	Barcelona, Spain	FJ986182
Loxosceles rufescens	Siles, Jaen, Spain	FJ986188
Loxosceles rufescens	Sierra Gorda, Cartagena, Murcia, Spain	FJ986180
Loxosceles rufescens	Alacant, Spain	FJ986184
Loxosceles rufescens	Testour, Tunisia	FJ986186
Dysdera crocata	Hoz de Pergrina, Guadalajara, Spain	EF458137

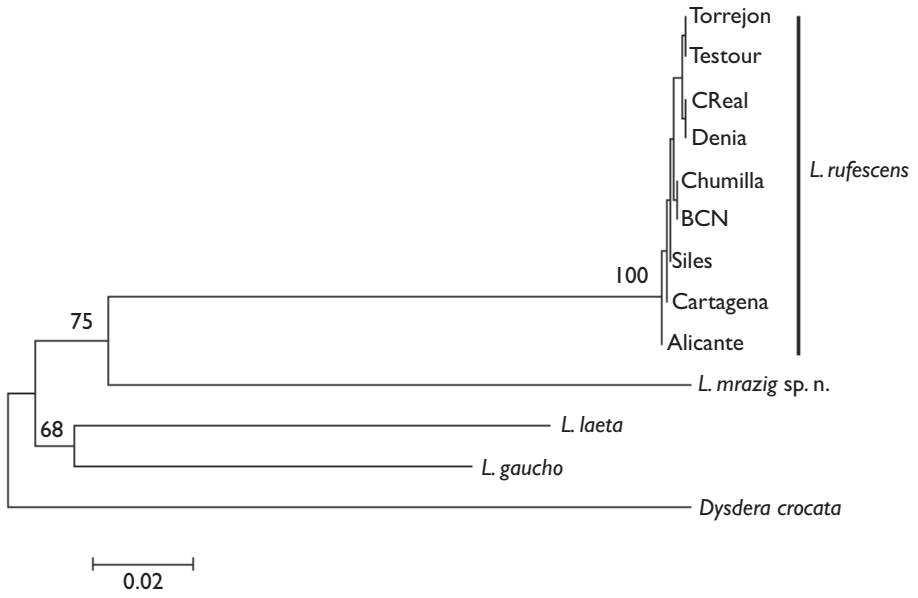


Figure 1. Neighbour-joining distance tree. Different representatives of *L. rufescens* from the western Mediterranean basin (Spain and Tunisia), *L. mrazig* sp. n., *L. gaucho* and *L. laeta* are included. Numbers on nodes represent bootstrap support values.

Taxonomy

Family Sicariidae
Genus *Loxosceles* Heineken et Lowe, 1832

***Loxosceles mrazig* sp. n.**
urn:lsid:zoobank.org:act:A8F75878-85CA-4567-874B-EBC8CC24CD02
Figs 2-7

Material examined. 1 male (Holotype) from Douz, Tunisia, 33° 24' 26.77" N, 09°02'41.92"E, 27 January 2007, Cesc Múrria leg. (CRBA-LX1054).

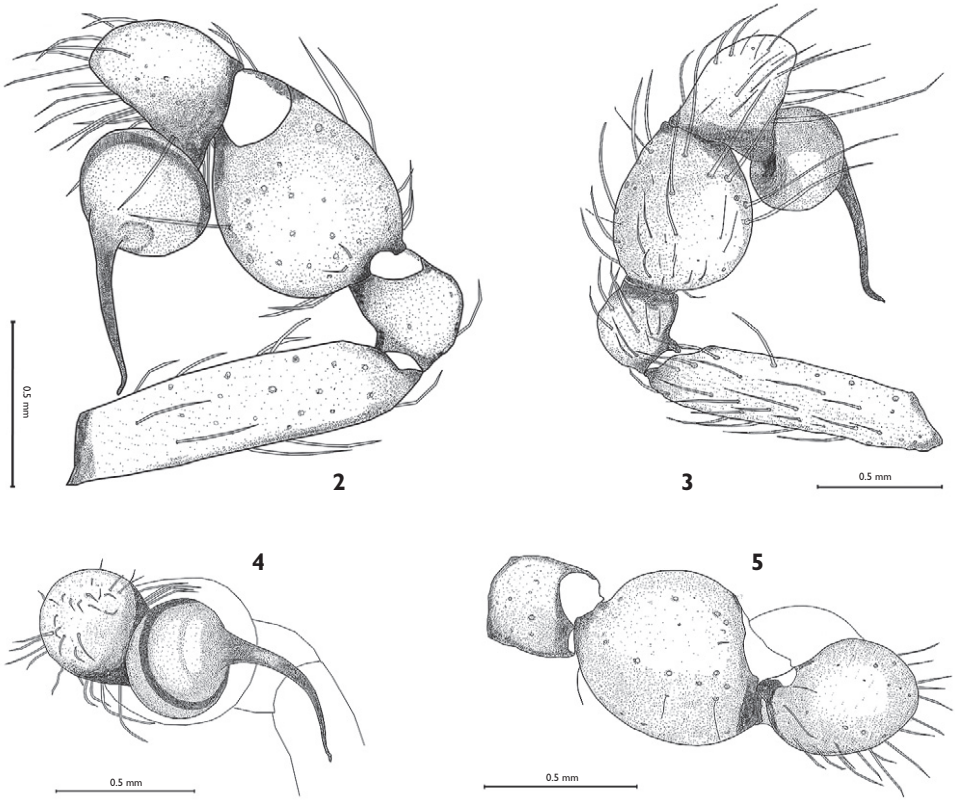
Material for comparison. 2 males, 2 females of *L. gaucho* (CRBA-LX1024) from Sao Paulo, Brazil, November 2007, A. Brescovit leg.; 2 males, 2 females of *L. laeta* (CRBA-LX1028) from Montevideo, Uruguay, L. Acosta leg.; 1 male, of *L. rufescens* (CRBA-LX1012) from Gavà, Barcelona, López-Pancorbo leg.

Etymology. The species' name honours the people called Mrazig, formerly nomadic, living in and around the city of Douz (Tunisia). The Mrazig are the descendants of the Banu Saleim tribe that fled the Arabian Peninsula in the seventh century and came to Tunisia in the thirteenth century. It is known that they practiced transhumance in the Great Sahara. Noun in apposition.

Diagnosis. Differs from *L. gaucho*, *L. rufescens* and its similar relatives in the proportion of male palp segments, mainly the tibia. In *L. mrazig* the tibia is markedly oval, slightly longer than wide (0.63 - 0.54) (Figs 2, 3, 5); in *L. gaucho* it is ¾ as wide as long (Gertsch 1967, plates 3-4), whereas, in *L. rufescens*, it is slightly oval, although dorsally almost straight (Gertsch 1967, plate 10). Also differs from *L. rufescens* by the size of the tegulum and the size and shape of the embolus (Figs 2-5). Body pigmentation yellowish-brown in *L. gaucho* and pale yellow in *L. mrazig*. In general, the morphological differences compared to *L. rufescens* are more conspicuous. The size of the tegulum and, especially, the shape and length of the embolus are clearly different.

Description. Colouration: Carapace pale yellowish with a fine, pale brown lateral stripe. Median groove and adjacent integuments darkened. Pars cephalica slightly darkened, brown coloured, and clearly demarcated by a lateral reddish brown line. Less conspicuous, but still important, diagnostic traits are the four thin longitudinal lines (lightly impressed when seen under higher magnification) located in the centre of the pars cephalica (Fig. 6). Eye tubercles black. Sternum pale yellowish, paler than carapace. Labium and gnathocoxae with slightly more pigmentation. Legs light yellow or somewhat shaded, with the apical segments slightly darkened. Opisthosoma yellowish-white.

Prosoma. Carapace (Fig. 6) slightly longer (2.39) than wide (2.15). Median groove deep, occupying the posterior third of carapace. Clypeal width slightly more than 2.5 diameters of ME. Eyes close together (Fig. 7); LE separated from ME by the diameter of ME. LE larger than ME (0.18 - 0.1 respectively). Sternum about ⅔ as wide as long, extended between the IV pair of coxae. Labium as long as wide at its base, apically narrowed and rounded. Gnathocoxae distally convergent, enclosing the labium.



Figures 2-5. Male palp of *Loxosceles mrazig* sp. n. **2** prolateral view **3** retrolateral view **4** apical view **5** dorsal view.

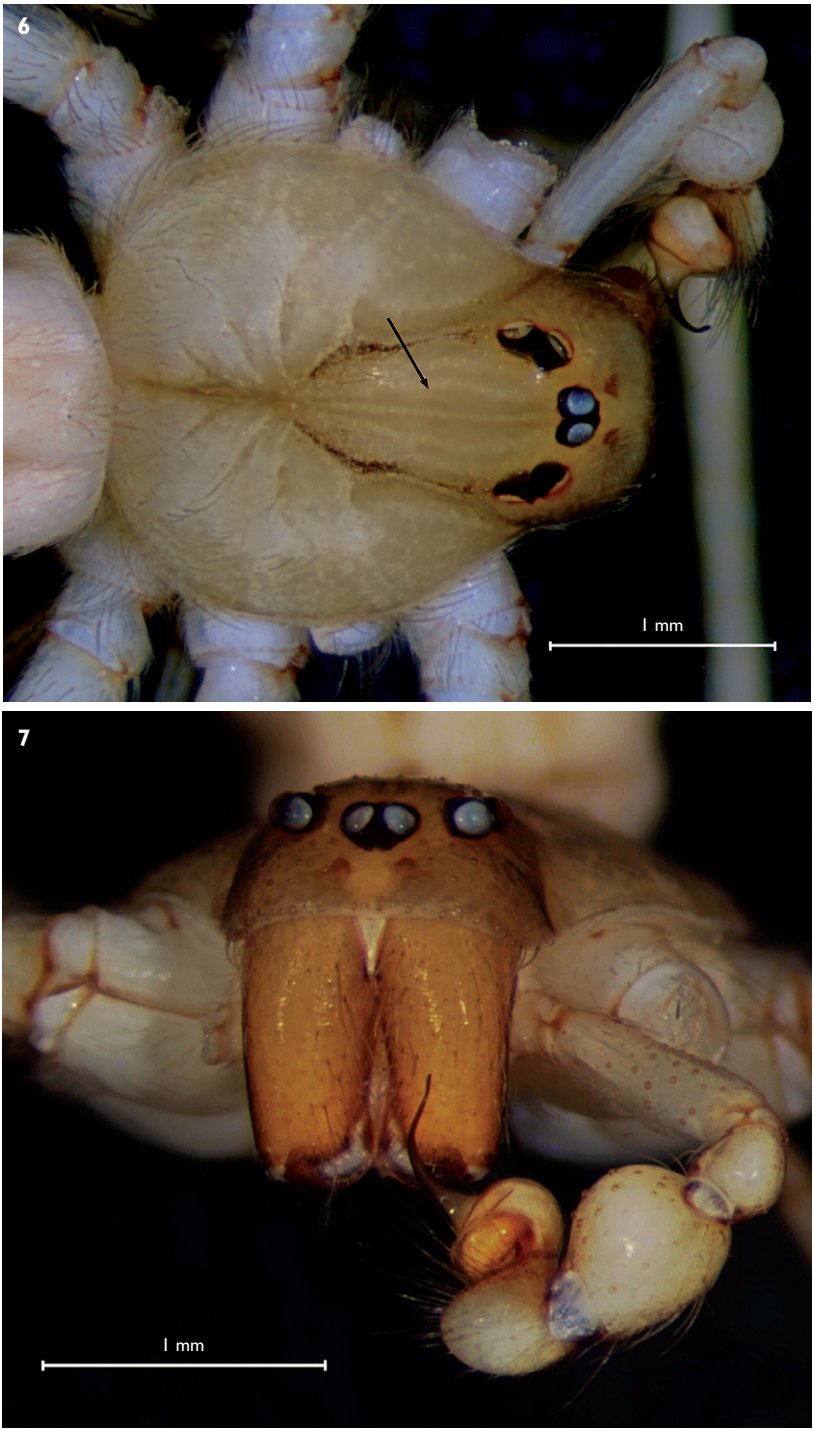
Opisthosoma elongate oval in dorsal view.

Male palp (Figs 2-5). Femur cylindrical, more than five times longer than wide. Tibia short, oval, slightly longer than wide. Tarsus flattened below, slightly shorter than tibia, rounded apically. Tegulum large, 4/5 as wide as tarsal length. Embolus enlarged at base, forming a sinuous curve, about 1.5 times longer than tegulum.

Measurements. Male (holotype): Prosoma 2.15 wide, 2.39 long: opisthosoma 3.22 long. Total body length 5.61. Legs: I: coxa 0.81, trochanter 0.23, femur 5.42, patella 0.84, tibia 5.70, metatarsus 5.76, tarsus 1.38, total length 20.14; II: coxa 0.58, rest of segments missing. III: coxa 0.81, trochanter 0.23, femur 4.94, patella 0.82, tibia 4.69, metatarsus 5.37, tarsus 1.12, total length 17.98; IV: coxa 0.81, trochanter 0.23, femur 5.26, patella 0.84, tibia 5.40, metatarsus 6.42, tarsus 1.33, total length 20.29; Palp: femur 1.19, patella 0.36, tibia 0.63, tarsus 0.56, total length 2.74.

Female unknown.

Distribution. So far, *L. mrazig* is known only from the type locality. The unique specimen was collected in a dune of sand near the city of Douz.



Figures 6-7. Prosoma of *Loxosceles mrazig* sp. n. **6** dorsal view, arrow indicates the four longitudinal lines located in the centre of the pars cephalica **7** frontal view.

Discussion

The possibility that this species should be assigned to *L. gaucho* can be ruled out due to the high genetic distance observed between both species (more than 20%) and especially because they do not form a sister group relationship, but belonging to different clades. The morphological similarity can be explained as a convergence phenomenon due to the simple morphological structures of the copulatory organs found in haplogyne spiders.

Determining the closest relatives is difficult for this species due to the lack of current knowledge on African *Loxosceles* species. Taking into account the shape of the male bulb, this species could be related to *L. foutadjalloni* Millot, 1941 from Guinea, in which the proportional palpal segments differs notably (mainly the tibia) and by the shape and size of the embolus. *L. mrazig* sp. n. – which could possibly be a member of a different group, or form a subgroup with the above mentioned *L. foutadjalloni*. *L. mrazig* sp. n. – is the second *Loxosceles* species known from the Mediterranean basin.

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